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Art Unit:	1648

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

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Helen C. Lockhart Ph.D., Reg No. 39,248

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Sir:

DECLARATION OF DR. HEATHER L. DAVIS UNDER 37 CFR §1.32

I, Dr. Heather L. Davis, declare as follows:

1. I make this Declaration in support of U.S. Serial No. 10/023,909 on which I am named as an inventor. I am the Vice President of Pharmacology Research and Development and Managing Director of Coley Pharmaceutical Group, Ltd., in Ottawa, Ontario, Canada.

2. I have been performing research on CpG immunostimulatory nucleic acids for several years.

3. Experiments performed in my laboratory have demonstrated that CpG oligonucleotides administered in combination with other immune stimulating adjuvants are effective in producing a synergistic immune response when delivered by a variety of administration routes. Our presented data confirms and is consistent with the teachings found in the specification that administration of a composition of a synergistic combination of CpG oligonucleotide and a variety of immune-stimulating adjuvants results in a significant Th1 response. I present a sampling of these findings below.

4. CpG oligonucleotides alone and with other adjuvants have been delivered orally, nasally, intramuscularly and subcutaneously to produce both mucosal and systemic immune responses. The data presented in Exhibit 1 demonstrate that oral, intramuscular, subcutaneous, and intranasal delivery of

hepatitis B surface antigen, HBsAg, with oligonucleotides in combination with an immune stimulating adjuvant induced synergistic amounts of antibody production over delivery of the antigen with either the oligonucleotide or the adjuvant alone.

5. Increase in IgG2a isotypes demonstrated in the data of Exhibit 1 is representative of activation of a Th1 immune response. The activation of Th1 response was further demonstrated by measurement of IFNγ production from isolated splenocytes of immunized animals. Re-stimulation of splenocytes with CpG and MF59 resulted in a dramatic increase of IFNγ production from cells.

6. An experiment was conducted to demonstrate the synergistic increase in antibody production when combinations of both depo and immune-stimulating adjuvants were administered in combination with CpG ODN. Figure one shows a bar graph depicting the effect of different adjuvants on total IgG titers of anti-HBs, wherein BALB/c mice (n=10/group) were immunized with 1 μg HBsAg alone or in combination with; (i) alum (25 mg AL3+); (ii) FIA (1:1 v/v); (iii) CpG ODN 1826 consisting of SEQ ID NO:86 with a phosphorothioate backbone (10 μg/animal); (iv) CpG ODN + alum; or (vi) CpG ODN + FIA. Animals were bled at 4 weeks post prime and plasma assayed for total IgG levels against HBsAg (Anti-HBs). Each bar represents the geometric mean (± SEM) of the ELISA end point dilution titer of anti-HBs total IgG for the entire group (n=10). Titers were defined as the highest dilution resulting in an absorbance value two times that of non-immune plasma with a cut-off value of 0.05.

7. An experiment was performed to measure the effects of Montanide (an adjuvant with both immune stimulating activity and depo effect) and CpG ODN on anti-HBS IgG titers in blood. Figure 2 of Exhibit 1 shows a bar graph depicting the effect Montanide ISA 720 (Seppic Inc., Paris, France) on total IgG titers (panel A) or IgG subtypes (panel B) of anti-HBS, wherein BALB/c mice (n=10/group) were immunized with 1 μg HBsAg alone or in combination with; (i) CpG ODN 1826 consisting of SEQ ID NO:86 with a phosphorothioate backbone (10 μg/animal); (ii) non-CpG control ODN 1982 (5' TCCAGGACTTCTCTCAGGTT 3') (10 μg/animal); (iii) Montanide ISA 720 (70:30 v/v of Montanide:antigen); (iv) CpG ODN 1826 + Montanide (70:30 v/v Montanide:antigen+ CpG ODN) or (v) ODN 1982 + Montanide (70:30 v/v Montanide:antigen+ ODN). Each bar represents the group geometric mean titers (GMT) (±SEM) for HBsAg-specific Antibodies (anti-HBs, IgG, IgG1, IgG2a) in plasma taken 4 wk post immunization. Titers were defined as the highest dilution resulting in an absorbance value two times that of non-immune plasma with a cut-off value of 0.0.

8. An experiment was performed to measure the effects of Montanide (an adjuvant with both immune stimulating activity and depo effect) and CpG ODN on anti-tetanus toxin IgG titers in

blood. Figure 3 of Exhibit 1 shows a bar graph depicting the effect Montanide ISA 720 on total IgG titers (panel A) or IgG subtypes (panel B) of anti-HBS, wherein BALB/c mice (n=10/group) were immunized with 10 μg Tetanus toxoid alone or in combination with; (i) CpG ODN 1826 (SEQ ID NO:86) with a phosphorothioate backbone (10 μg/animal); (10 μg/animal); (ii) non-CpG control ODN 1982 (10 μg/animal); (iii) Montanide ISA 720 (70:30 v/v of Montanide:antigen); (iv) CpG ODN 1826 + Montanide (70:30 v/v Montanide:antigen+ CpG ODN) or (v)) ODN 1982 + Montanide (70:30 v/v Montanide:antigen+ ODN). Each bar represents the group geometric mean titers (GMT) (±SEM) for HBsAg-specific Antibodies (anti-HBs, IgG, IgG1, IgG2a) in plasma taken 4 wk post immunization. Titers were defined as the highest dilution resulting in an absorbance value two times that of non-immune plasma with a cut-off value of 0.05

9. An experiment was performed to demonstrate the synergistic production of anti-HBs antibody after stimulation with CpG ODN and Cholera toxin (CT), an immune stimulating adjuvant. Figure 4 of Exhibit one is a bar graph depicting the effect of different adjuvants on total IgG titers of anti-HBS, wherein BALB/c mice were immunized by IN inhalation with HBsAg (1 or 10 μg) without or in combination with Cholera toxin (CT) and/or CpG oligonucleotide (motif #1826, SEQ ID NO:86) adjuvants.

10. An experiment was performed to measure IgG ELISA titer after intranasal inhalation of HBsAg. Figure 5 of Exhibit 1 is a graph depicting the effect of different adjuvants on total IgG titers of anti-HBs, wherein BALB/c mice were immunized by IN inhalation with HBsAg (1 μg) without or in combination with Cholera toxin (CT, an immune stimulating adjuvant) and/or CpG oligonucleotide (motif #1826, SEQ ID NO:86) adjuvants and at 8 weeks mice were boosted in the same manner as prime.

11. An experiment was performed to measure ELISA IgG isotypes after intranasal inhalation of HBsAg. Figure 6 of Exhibit 1 is a bar graph depicting the effect of different adjuvants on anti-HBs IgG isotype, wherein BALB/c mice were immunized by IN inhalation with HBsAg (1 μg) without or in combination with Cholera toxin (CT) and/or CpG oligonucleotide (motif #1826, SEQ ID NO:86) adjuvants (1 μg) and at 8 weeks mice were boosted in the same manner as prime.

12. An experiment was performed on isolated splenocytes of immunized mice to measure response to cytotoxic T-cell lymphocytes (CTL) of the cultured splenocytes. Figure 7 of Exhibit 1 is a bar graph depicting the effect of different adjuvants on HBsAg specific CTL response, wherein BALB/c mice were immunized by IN inhalation with HBsAg (10 μg) without or in combination with Cholera toxin (CT) and/or CpG oligonucleotide (motif #1826, SEQ ID NO:86) adjuvants at different doses (1 or

10 μg) and four weeks after immunization mice were killed by Halothane overdose, splenocytes isolated and HBsAg specific CTL activity measured.

13. An experiment was performed to measure the total anti-HBs IgA ELISA titer in mice immunized by inhalation. Figure 8 of Exhibit 1 is a bar graph depicting the effect of different adjuvants on anti-HBs IgA titers in lung washes, wherein BALB/c mice were immunized by IN inhalation with HBsAg (1 or 10 μg) without or in combination with Cholera toxin (CT) and/or CpG oligonucleotide (motif #1826, SEQ ID NO:86) adjuvants at different doses (1 or 10 μg) and four weeks after immunization (or after boost for group marked by *) mice were killed by Halothane overdose and lungs were washed with 1ml TBS.

14. An experiment was performed to measure HBs IgA titers in fecal pellets of mice immunized by inhalation. Figure 9 of Exhibit 1 is a bar graph depicting the effect of different adjuvants on anti-HBs IgA titers in fecal pellet solutions, wherein BALB/c mice were immunized by IN inhalation with HBsAg (1 or 10 μg) without or in combination with Cholera toxin (CT) and/or CpG oligonucleotide (motif #1826, (SEQ ID NO:86)) at different doses (1 or 10 μg) and four weeks after immunization (or after boost for group marked by *) mice were isolated for 24 hr and fecal pellets were collected and resuspended in TBS at 0.1 mg/ml.

15. An experiment was performed to measure the effect of different adjuvants on total IgG titers of anti-HBs in mice. Figure 10 of Exhibit 1 is a bar graph depicting the effect of different adjuvants on total IgG titers of anti-HBs, wherein BALB/c mice were immunized by IN inhalation with HBsAg (1 μg) without or in combination with Cholera toxin (CT), *Escherichia coli* heat-labile enterotoxin (LT), the B subunit of Cholera toxin (CTB), a detoxified mutant of *Escherichia coli* heat-labile enterotoxin (LTK63), CpG oligonucleotide (motif #1826, SEQ ID NO:86) or non-CpG control oligonucleotide (motif #1982, 5' TCCAGGACTTCTCTCAGGTT 3') adjuvants (1, 10 or 500 μg). In groups which responded, all mice gave titers > 10, except in the case of 10 μg LT where only 1/5 mice responded.

16. An experiment was performed to measure the effect of different adjuvants with and without CpG ODN on total anti-HBs or anti-tetanus toxoid (TT) antibody responses in mice. Figure 11 of Exhibit 1 is a bar graph depicting the effect of different adjuvants on total antigen-specific IgG titers, wherein BALB/c mice were immunized by IN inhalation of 10 μg HBsAg (panel A) or tetanus toxoid (TT) (panel B) either alone (none) or with 1 μg (white bars) or 10 μg (gray bars) of CpG oligodeoxynucleotides (motif #1826, (SEQ ID NO:86)), *Escherichia coli* heat-labile enterotoxin (LTh), the B subunit of *Escherichia coli* heat-labile enterotoxin (LTB), or various detoxified mutant of *Escherichia coli* heat-labile enterotoxin (LTE112K, LTS61F, LTR192G, or LTA69G) as adjuvant. In

addition, other mice were immunized with CpG combined with one of the other adjuvants (1 μg each, black bars). Mice were boosted in an identical manner at 4 and 8 weeks. Each bar represents the group mean (± SEM) of the ELISA titer for HBsAg-specific (anti-HBs, total IgG) or TT-specific (anti-TT, total IgG) antibodies in plasma taken 4 weeks after third and final immunization.

17. An experiment was performed to determine the effect of different adjuvants on total antigen-specific IgA titers of mice immunized against HBsAg or TT by intranasal (IN) administration. Figure 12 of Exhibit 1 is a bar graph depicting the results wherein BALB/c mice (n = 5) were immunized by IN delivery of 10 μg HBsAg (panels A-D) or TT (panels E-H) either alone (none) or with 1 μg (white bars) or 10 μg (gray bars) of CpG oligodeoxynucleotides (motif #1826, (SEQ ID NO:86)), *Escherichia coli* heat-labile enterotoxin (LTh), the B subunit of *Escherichia coli* heat-labile enterotoxin (LTB), or various detoxified mutant of *Escherichia coli* heat-labile enterotoxin (LTE112K, LTS61F, LTR192G, or LTA69G) as adjuvant.. In addition, other mice were immunized with CpG combined with one of the other adjuvants (1 μg each, black bars). Mice were boosted in an identical manner at 4 and 8 weeks. Panels A, B, E and F: Each bar represents the group mean (± SEM) of the ELISA titer for HBsAg-specific (anti-HBs IgA GMT) or TT-specific (anti-TT IgA GMT) antibodies in mucosal samples (lung, or gut washes) taken 4 weeks after third and final immunization. Panels C, D, G and H: Each bar represents the ELISA titer for HBsAg-specific (anti-HBs IgA) or TT-specific (anti-TT IgA) antibodies in pooled mucosal samples (vaginal wash or saliva) taken 4 weeks after third and final immunization.

18. An experiment was performed in mice to determine the effect of different adjuvants on total antigen-specific IgG titers against TT, which was administered by oral delivery. Figure 13 is a bar graph depicting the results wherein BALB/c mice (n = 5) were immunized by oral delivery on days 0, 7, 14 with 10 μg TT either alone (none) or with 10 μg of CpG oligodeoxynucleotides (motif #1826, (SEQ ID NO:86)), *Escherichia coli* heat-labile enterotoxin (LTh), the B subunit of *Escherichia coli* heat-labile enterotoxin (LTB), or various detoxified mutant of *Escherichia coli* heat-labile enterotoxin (LTE112K, LTS61F, LTR192G, or LTA69G) as adjuvant. In addition, other mice were immunized with CpG combined with one of the other adjuvants (1 μg each).

Panel A: Each bar represents the group mean (± SEM) of the ELISA titer for TT-specific (anti-TT, total IgG) antibodies in plasma taken 1 wk after third and final immunization.

Panel B: Each bar represents the group geometric mean (± SEM) of the ELISA titer for TT-specific antibodies of IgG1 (gray bars) or IgG2a (black bars) isotypes in plasma taken 1 week after final immunization.

19. An experiment was performed to determine the effect of different immune-stimulating adjuvants with and without CpG ODN on total salivary IgA in mice immunized against TT by oral delivery. Figure 14 of Exhibit 1 is a bar graph depicting the effect of different adjuvants on total antigen-specific IgA saliva titers, wherein BALB/c mice (n = 5) were immunized by oral delivery on days 0, 7, 14 with 10 μg TT either alone (none) or with 10 μg of CpG oligodeoxynucleotides (motif #1826, (SEQ ID NO:86)), *Escherichia coli* heat-labile enterotoxin (LTh), the B subunit of *Escherichia coli* heat-labile enterotoxin (LTB), or various detoxified mutant of *Escherichia coli* heat-labile enterotoxin (LTE112K, LTS61F, LTR192G, or LTA69G) as adjuvant. In addition, other mice were immunized with CpG combined with one of the other adjuvants (1 μg each). Each point (panels A, B) represents the ELISA titer for Ag-specific IgA antibodies in lung and gut washes of individual animals, and bars (panels C, D) represent the ELISA titer for Ag-specific IgA antibodies in pooled vaginal washes and saliva.

20. An experiment was performed to measure total IgG and the IgG2a/IgG1 subtype ratio of antibodies in mice immunized against HBsAg by intramuscular (IM) injection. Figure 15 of Exhibit 1 is a bar graph depicting the effect of different adjuvants on HBsAg-specific IgG responses, wherein BALB/c mice were immunized by IM injection with HBsAg (1 μg) without or in combination with MF-59 and/or 10 μg CpG oligonucleotide (motif #7909, SEQ ID NO:77) adjuvants.

21. An experiment was performed to measure the effect of different immune-stimulating adjuvants with or without CpG ODN on HBsAg-specific CTL activity in mice immunized by intramuscular injection. Figure 16 of Exhibit 1 is a graph depicting the effect of different adjuvants on HBsAg-specific CTL activity, wherein BALB/c mice were immunized by IM injection with HBsAg (1 μg) without or in combination with MF-59 and/or 10 μg CpG oligonucleotide (motif #7909, SEQ ID NO:77) adjuvants.

22. An experiment was performed to measure the effect of different immune-stimulating adjuvants, with or without CpG ODN, on HBsAg-specific IFN-γ secretion from splenocytes of mice. Figure 17 of Exhibit 1 is a bar graph depicting the effect of different adjuvants on IFN-γ secretion following antigen re-stimulation of splenocytes from immunized animals, wherein BALB/c mice were immunized by IM injection with HBsAg (1 μg) without or in combination with MF-59 and/or 10 μg CpG oligonucleotide (motif #7909, SEQ ID NO:77) adjuvants.

23. An experiment was performed to measure the effect of different adjuvants, with or without CpG ODN, on total IgG titers or titers of IgG subtypes against HBsAg in mice. Figure 18 of Exhibit 1 is a bar graph depicting the effect of different adjuvants on total IgG titers (panel A) or IgG subtypes (panel B) of anti-HBS, wherein BALB/c mice were immunized by subcutaneous (SC) injection

on days 0, 28 with HBsAg (1 μg) without or in combination with 10 μg CpG ODN (motif #7909, SEQ ID NO:77) and/or 143.75 μg calcium phosphate nanoparticles (CAP) as adjuvant. Each bar represents the group geometric mean titers (GMT) (±SEM) for HBsAg-specific Abs (anti-HBs, IgG, IgG1, IgG2a) in plasma taken 4 wk after second immunization. Titers were defined as the highest dilution resulting in an absorbance value 2X that of non-immune plasma.

24. I, Dr. Heather L. Davis, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

Date: October 25, 2004 By: HL Davis

Dr. Heather L. Davis